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Effect of PEF, HHP and thermal treatment on PME inactivation and volatile compounds concentration of an orange juice–milk based beverage ☆

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ABSTRACT

The effects of thermal, pulsed electric field (PEF) and high hydrostatic pressure (HHP) processing on pectin methyl esterase (PME) activity and volatile compounds concentration in an orange juice–milk beverage were studied. Thermal treatment (85 °C, 1 min), PEF treatment (25 kV/cm, 65 °C) or HHP treatment (650 MPa, 50 °C) were needed to inactivate 90% of PME. Twelve volatile compounds were extracted by solid-phase microextraction (SPME) and selected for quantification by GC-MS following the application of the different treatments. The average loss in concentration of volatile compounds was between 16.0 and 43.0% after thermal treatment. After PEF treatment the average loss was between −13.7 and 8.3% at 25 °C, 5.8 and 21.0% at 45 °C and 11.6 and 30.5% at 65 °C. After HHP treatment the average loss was between −14.2 and 7.5% at 30 °C and 22.9 and 42.3% at 50 °C. The results showed the potential of the nonthermal technologies in providing food with a higher standard of quality compared to thermal processing.

Industrial relevance: The use of nonthermal technologies as an alternative to heat processing in the pasteurisation of beverages has acquired relevance in the last years. In this manuscript, we have shown that PEF treatment could achieve a high degree of PME inactivation in an orange juice based beverage, while better preserving the natural aroma than HHP and thermal treatments. PEF processing has an enormous potential to pasteurise fruit juice and preserve its natural quality characteristics.

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1. Introduction

The use of High Hydrostatic Pressure (HHP) as a food preservation technology is gaining relevance in recent years, especially in Japan where the use of HHP has been adopted for the preservation of fruit juices, jams, sauces, rice, cakes and desserts (Norton & Sun, 2008). Nowadays in Europe and the USA, several companies in the meat, dairy and beverage industry have commercialized HHP treated products and it is thought that this trend will increase (Trujillo, Capellas, Saldo, Gervilla, & Guamis, 2002). Regarding the use of Pulsed Electric Fields (PEF), different research groups are working on the use of PEF for preservation of fruit and vegetable juices, milk and liquid egg (Sampedro, Rodrigo, Martínez, Rodrigo, & Barbosa-Cánovas, 2005; Rodrigo, Sampedro, Martínez, Barbosa-Cánovas, & Rodrigo, 2005; Sampedro, Rodrigo, Martínez, Rodrigo, & Barbosa-Cánovas, 2006). The use of PEF by the juice processing industry became a reality in the USA where several types of fruit juices treated by PEF were commercialized (Clark, 2006); however, the PEF treated juice is no longer being produced.

A large quantity of minimally processed foods have appeared on the market with characteristics similar to the original fresh food in response to a growing demand for natural foods that are perceived by consumers as healthy. Among them are beverages based on a mix of fruit juices and milk fortified with vitamins, minerals, and fiber as the most widely consumed functional foods (Pszczola, 2005); however, there are limited data related to quality and safety of these products.

Pectin Methyl Esterase (PME) is an important enzyme in orange juice based products and PME spoilage effects in orange juice are well known as cloud loss or gelification of juice concentrates (Tribess & Tadini, 2006). Thermal preservation treatments (88–95 °C, 15–30 s) are based on the PME inactivation level achieved (>90%) due to its higher thermotolerance than those of majority of microorganisms found naturally in these products (Irwe & Olson, 1994).

The analysis of the volatile compounds has been performed by several authors to study the effect of PEF, thermal and HHP treatment on orange juice aroma (Jia, Zhang, & Min, 1999; Yeom, Streaker, Zhang, & Min, 2000; Ayhan, Zhang, & Min, 2002; Min, Jin, Min, Yeom, & Zhang, 2003; Baxter, Easton, Schneebeli, & Whitfield, 2005) but there is no study on the effect of PEF and HHP treatment on the volatile compounds in a mixture of orange juice and milk.

The aim of this work was to study the effects of PEF, HHP and thermal processes on PME activity and volatile compounds in an orange juice–milk based beverage (OJMB).

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2. Materials and methods

2.1. Beverage preparation

Fresh Valencia var. oranges were purchased at a local supermarket. The oranges were squeezed with a juice extractor (Zumex 38, Zumex, S.A., Spain) and the juice was filtered with cheese cloth and stored at −40 °C. The OJMB contained the following ingredients: fresh orange juice (500 mL/L), commercial UHT skimmed milk (200 mL/L), high methoxyl citrus pectin (Degussa Food Ingredients, Spain) (3 g/L), sucrose (75 g/L), and deionized water (300 mL/L). Prior to mixing, solid ingredients were dissolved in water in the weight proportions indicated. The beverage was prepared just before use. The OJMB physicochemical characteristics were reported in a previous article (Sampedro, Rivas, Rodrigo, Martínez, & Rodrigo, 2007).

2.2. Thermal treatment

Inactivation experiments were carried out in a water bath with temperature control, in a range from 60 to 90 °C for 1 min. One mL of sample was enclosed in a 1 mL thermal death time (TDT) disk (Jin, Zhang, Boyd, & Tang, 2008). The samples were pre-heated to 40 °C (results indicated that no PME inactivation was produced at this temperature, data not shown) in order to shorten and standardize the come-up time. The time needed to reach the final temperatures from the preheating temperature of 40 °C was about 1 min. After treatment at the preset temperatures, the samples were withdrawn from the water bath and immediately cooled and kept in ice-water. The residual PME activity was measured within 2 h.

2.3. PEF treatment

An OSU-4F bench-scale continuous unit (Ohio State University, USA) was used to provide PEF treatment (Table 1). Six co-field chambers with a diameter of 0.23 cm and a gap distance of 0.29 cm between electrodes were connected in series. One cooling coil was connected before and after each pair of chambers and submerged in a circulating bath (model 1016 S, Fisher Scientific, PA, USA) to maintain the selected temperature. The temperature was recorded by thermocouples (K type) at the entrance and exit of each pair of chambers. The entrance of the first treatment chamber can be considered as the initial temperature and the exit of the last treatment chamber as the final temperature. The values were recorded with a data logger (Model 800024, Sper Scientific, Taiwan). Pulse waveform, voltage, and current in the treatment chambers were monitored with a digital oscilloscope (Tektronix TDS 210, USA). The flow rate was controlled with a digital gear pump (Model 75211-30, Cole Parmer, USA). One sample was collected after each treatment time and immediately cooled in ice-water.

Table 1
PEF process conditions (OSU 4-F).

Description	Operating conditions
Electric field strength (kV/cm)	15–30
Peak current (A)	11.2–22.4
Polarity	Bipolar
Wave shape	Square wave
Pulse duration (μs)	2.5
Repetition rate (pps)	547
Number of chambers	6
Flow rate (mL/min)	120
Number of pulses per chamber	3.3
Total treatment time (μs)	50
Initial temperature (°C)	25
	45
	65
Final temperature at highest electric field (°C)	47.1[a] ± 2.3[b]
	62.5 ± 2.7
	80.3 ± 2.1
Back pressure (bar)	1.72

[a] Value based on mean of three replicates.
[b] Standard deviation.

Table 2
HHP process conditions (2 L model).

Description	Operating conditions
Pressure range (MPa)	450–650
Come-up time (min)	2
Holding time (min)	15
Initial temperature (°C)	30
	50
Final temperature after pressurization at highest pressure (°C)	44.7[a] ± 2.5[b]
	68.0 ± 1.9
Final temperature after holding time at highest pressure (°C)	34.9 ± 1.3
	55.3 ± 1.7
Final temperature after decompression at highest pressure (°C)	18.3 ± 1.2
	39.9 ± 2.1

[a] Value based on mean of three replicates.
[b] Standard deviation.

2.4. HHP treatment

All pressure experiments were performed in a laboratory-scale vessel high-pressure processor (model 2L, Autoclave Systems Inc., USA) (Table 2). The pressure medium was deionized water. A thermostated mantel, which surrounded the vessel, was connected to a cryostat keeping the vessel wall temperature constant during the experiment. Temperature was recorded by a thermocouple placed inside the vessel. The samples were filled in 2 mL eppendorf tubes and were enclosed in the pressure vessel already equilibrated at an initial temperature. The vessel was then pressurized and after a preset hold-time, decompressed. After pressure release the samples were immediately cooled in ice-water.

2.5. Analysis of headspace volatile compounds

Volatile compounds were extracted with a modification of the method described by (Fan & Gates, 2001) using a solid-phase microextraction (SPME) method. A 2 mL aliquot beverage was transferred into 6 mL serum vial. The vial, sealed by a teflon-lined septum and a screw cap, was pre-heated at 60 °C for 2 min before a SPME fiber, coated with 100 μm of poly(dimethylsiloxane), was inserted into the headspace of the vial. After 30 min incubation, the SPME fiber with adsorbed volatile compounds was removed from the vial and inserted into the GC injection port at 250 °C and held there for 5 min to desorb volatile compounds. Volatile compounds were separated by a Hewlett-Packard 6890 N/5973 GC-MSD (Agilent Technologies, USA) equipped with a DB-Wax trace analysis column (30 m × 0.32 mm i.d., 0.5 μm film thickness). The temperature of the GC was programmed from 60 to 96 °C at 8 °C·min^{-1}, increased to 120 °C at 12 °C·min^{-1}, then increased to 220 °C at 10 °C·min^{-1} and held for 3 min at the final temperature. Helium was the carrier gas at a linear flow speed of 39 cm·s^{-1}. Compounds were identified by comparing spectra of the sample with those contained in the National Institute of Standards and Technology Library (NIST02). The relative amount of each compound was expressed as peak area.

2.6. PME activity measurement

PME activity was determined by measuring the release of acid over time at pH 7 and 22 °C following the procedure described by (Van den Broeck, Ludikhuyze, Weemaes, Van Loey, & Hendrickx, 1999). The reaction mixture consisted of 1 mL of sample and 30 mL of 0.35% citrus pectin solution (Sigma, USA) containing 125 mM NaCl. During hydrolysis at 22 °C, pH was maintained at 7.0 by adding 0.0001 N

NaOH using an automatic pH-stat titrator (Titralab, Radiometer Analytical, SAS). After the first 1 min the consumption of NaOH was recorded every 1 s for a 3 min reaction period. PME activity was expressed in units (U/mL), defined as micromoles of acid produced per minute at pH 7 and 22 °C. The detection limit was established at 0.019 U/mL. In order to account for the effect of come-up time on the enzyme activity, residual activity was expressed as the relation between the PME activity after the treatment (A) expressed in U/mL and the initial activity (A_0) expressed also in U/mL.

2.7. Experimental design and statistical analysis

A hierarchical experimental design was used to study the influence of the different technologies: in thermal treatment, seven temperatures in PEF treatment, four electric field strengths and three temperatures and in HHP treatment, five pressures and two temperatures. The statistical analysis was performed using the Statgraphics software (Statistical Graphics Corp., USA), applying a univariant ANOVA test with a significance level of 95.0%.

3. Results and discussion

3.1. Effect of treatment on PME activity in the orange juice–milk based beverage

Fig. 1 shows the thermal inactivation of PME in the OJMB in the range of 60–90 °C for 1 min. In order to account the effect of come-up time on the enzyme activity, residual activity was expressed as the relation between the PME activity after the treatment (A) expressed in U/mL and the activity after the come-up time (A_{cut}). Approximately 4–5% of the PME remained after the 90 °C treatment. Several authors Versteeg, Rombouts, Spaansen, and Pilnik (1980); Wicker and Temelli (1988); Snir, Koehler, Sims, Wicker (1996); Cameron and Grohmann (1996); Tajchakavit and Ramaswamy (1997); Cameron, Baker, and Grohmann (1998); Lee, Lin, Chang, Chen, and Wu (2003); Do Amaral, De Assis, and De Faria Oliveira (2005) have also observed a residual activity after thermal treatment, considered the stable fraction, between 4 and 8.3% in Navel and Valencia var. oranges reflecting the thermotolerance of the orange PME. To achieve a level of 90% of PME inactivation, 85 °C for 1 min or 90 °C for 30 s was needed. No inactivation was found below 65 °C for 1 min. At 60 °C the PME activity was increased as a result of the treatment. This phenomenon could be explained by some activation effect produced by the heat. Körner, Zimmerman, and Berk (1980) found an optimum temperature of 60 °C for the activity of two PME fractions purified from Valencia flavedo and Shamouti juice. The relationship between the temperature and the residual activity of PME seemed to be linear from 60 to 80 °C and after that a tailing phenomenon was observed, suggesting the presence of a thermostable fraction in the OJMB.

Table 3 shows the combined thermal and PEF inactivation of PME in the OJMB in the range of 15–30 kV/cm and initial temperatures of 25–65 °C. At low treatment temperature (25 °C) some activation effect was found as indicated by an increase in PME activity (between 11 and 60%) after the PEF treatment. PEF treatment has also been applied in different studies in recent years to improve the extraction of different components by increasing the permeability of plant cells from diverse foodstuff (Ade-Omowaye, Angersbach, Taiwo, & Knorr, 2001) and by improving the juice yield and quality parameters (Guderjan, Elez-Martinez, & Knorr, 2007; Schilling et al., 2007). Therefore application of mild PEF treatments could increase the permeability of the orange pulp by facilitating the release of the bound PME. In those studies, after the PEF treatment, PME activity measured as "free" enzyme, increased. Van Loey, Verachert, and Hendrickx (2002) noticed that after PEF treatment, polyphenoloxidase in apple juice and PME in orange juice were activated due to increases in cell permeabilization and release of the enzyme from plant cells. In addition, some authors have also observed an enhancing effect of diverse enzymes after PEF treatment, such as alkaline phosphatase and peroxidase in milk (Grahl & Märkl, 1996), lysozyme and pepsin in buffer solutions (Ho, Mittal, & Cross, 1997), PME in orange juice (Yeom, Zhang, & Chism, 2002), pepsin in an aqueous solution (Yang, Li, & Zhang, 2004) and protease in milk (Bendicho, Marsellés-Fontanet, Barbosa-Cánovas, & Martín-Belloso, 2005). They argued that PEF treatment could create more active sites or increase the size of the existing sites converting the enzyme into a more active form.

Table 3
PME residual activity after combined thermal and PEF treatment in the orange juice–milk based beverage.

	Residual activity (A/A_0), initial T (°C)		
E (kV/cm)	25	45	65
15	1.594[a] ± 0.150[b]	0.947 ± 0.010	0.541 ± 0.012
20	1.484 ± 0.001	0.634 ± 0.032	0.482 ± 0.057
25	1.303 ± 0.001	0.642 ± 0.045	0.213 ± 0.048
30	1.118 ± 0.060	0.465 ± 0.021	0.089 ± 0.010

[a] Value based on mean of three replicates.
[b] Standard deviation.

By increasing the temperature, the inactivation reached a maximum of 91% inactivation after 30 kV/cm, 65 °C (final temperature 80 °C) and 50 μs. The residence time at this temperature was a few seconds. At these conditions the temperature could affect the enzyme to some extent. To check the thermal effect of the temperature in the PEF treatment, a treatment based on low electric field intensities, high frequency and low pulse duration was applied (3–5 kV/cm, 3000–3500 Hz, 1 μs) obtaining the same final treatment temperature (80 °C). Due to the low intensity PEF treatment only the thermal effect was observed. The results showed that slight PME inactivation (<10%) was achieved due only to the temperature demonstrating the synergetic effect between the temperature and PEF treatment.

Fig. 2 represents the combined thermal and HHP inactivation of PME in the OJMB at 30 and 50 °C for 15 min. At 25 °C and low pressures (450 MPa) no inactivation effect was observed, revealing the pressure tolerance of the orange PME. By increasing the temperature to 50 °C (final temperature 65 °C) and pressure to 650 MPa the inactivation increased and reached a maximum of 90.5%. Several authors have observed this resistance to pressure treatment. Nienaber and Shellhammer (2001) and

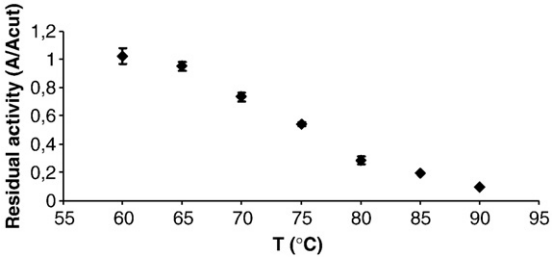

Fig. 1. Inactivation of PME in orange juice–milk based beverage for 1 min.

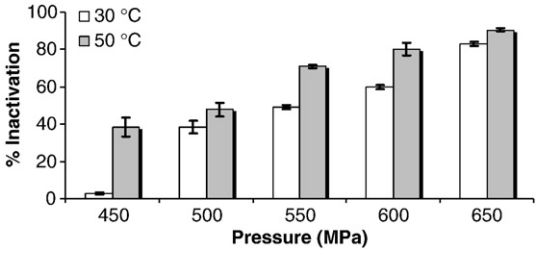

Fig. 2. Combined thermal and HHP inactivation of PME in orange juice–milk based beverage at two initial temperatures.

Truong, Boff, Min, and Shellhammer (2002) found that PME was stable under pressure below 400 MPa and combination of high temperature and high pressure (40–50 °C and 600–700 MPa) were necessary to inactivate it. From an industrial point of view, a higher pressure and/or temperature would be needed to shorten the holding time.

3.2. Effect of treatment on the volatile compounds concentration in the orange juice–milk based beverage

Twelve volatile compounds could be identified accurately from the OJMB. The majority of the compounds were hydrocarbons among which limonene and valencene were the most abundant constituting more than 90% of the total compounds.

Fig. 3 shows the effect of thermal treatment on the relative concentration of volatile compounds in the OJMB. The average loss of volatile compounds after thermal treatment at the different temperature range for 1 min was between 16.0 and 43.0%. There was a significant reduction ($p \leq 0.05$) in the concentration of volatile compounds due to increase in temperature but no significant difference was observed between 85 and 90 °C ($p > 0.05$). The compounds could be divided in two groups. One group considered as relatively low-molecular-weight (MW 136) including (β-pinene, α-pinene, β-myrcene, limonene, α-phellandrene, 3-carene and 4-carene) and a second group considered as relatively high-molecular-weight (MW 142-204) including nonanal, decanal, caryophyllene, dodecanal and valencene. The higher average loss was observed in β-pinene (48%), α-pinene (42%) and 3-carene (41%) which belong to the first group, and counted for an average loss of 35%. On the other hand the high-molecular-weight compounds seemed to be less sensitive to the thermal treatment with a slight increase in nonanal content at temperatures below 70 °C. At higher temperatures, nonanal and caryophyllene (12%) which have higher boiling points had an average loss of 21%, lower than the loss of lower boiling point compounds. Boff, Truong, Min, and Shellhammer (2003) came to the same conclusions observing that in the thermal processing of an orange juice, low-molecular-weight compounds (β-myrcene, limonene and α-pinene) were more sensitive to the thermal treatment than relatively higher-molecular-weight compounds (decanal, caryophyllene and valencene).

Fig. 4 shows the effect of the combined thermal and PEF treatment (at initial temperatures 25, 45 and 65 °C) on the concentration of volatile compounds in the OJMB. The average loss was between −13.7 and 8.3% at 25 °C, 5.8 and 21.0% at 45 °C and 11.6 and 30.5% at 65 °C after PEF treatment. An increase in the temperature and electric field strength produced a significant decrease ($p \leq 0.05$) in volatile compounds content. However at high electric field intensities (25 and 30 kV/cm) there were no differences between 45 and 65 °C ($p > 0.05$).

At 25 and 45 °C and electric field strength below 25 kV/cm and 65 °C at 15 kV/cm, several compounds increased its content after PEF treatment (β-pinene, limonene, 3-carene, 4-carene, nonanal, decanal and dodecanal). Ayhan et al. (2002) obtained similar results observing that several compounds (myrcene, limonene and α-pinene) increased its content after PEF. Steffen and Pawliszyn (1996) found that in a complex matrix such as orange juice, with the presence of suspended solids, a portion of analytes could be trapped in the pulp. PEF technology has been used to improve the extraction of different compounds by increasing the membrane permeabilization. This fact could explain why PEF treatment could facilitate the release of several compounds from the suspended solids to the liquid phase facilitating its extraction into the headspace.

To check the hypothesis of higher concentration of certain compounds in the pulp, the orange juice was centrifuged in order to separate into serum and pulp. The concentration of different compounds was studied in both fractions. Nonanal, 3-carene, 4-carene, limonene and α-phellandrene were found in higher quantity in the pulp (data not shown). This fact could explain that the compounds could be released into the liquid phase by the PEF treatment and be extracted in the headspace more readily.

Compounds more sensitive to the PEF treatment were β-myrcene, α-pinene and caryophyllene whereas the less sensitive compounds were limonene, 4-carene and nonanal. Opposite to the thermal treatment, the compounds that were present in the pulp at higher proportions were also higher after PEF treatment.

Fig. 5 shows the loss of volatile compounds concentration in the OJMB after the combined thermal and HHP (at 30 and 50 °C initial temperatures) treatment. The average loss at 30 °C was between −14.2 and 7.5%. Only β-myrcene content was lost at every pressure value

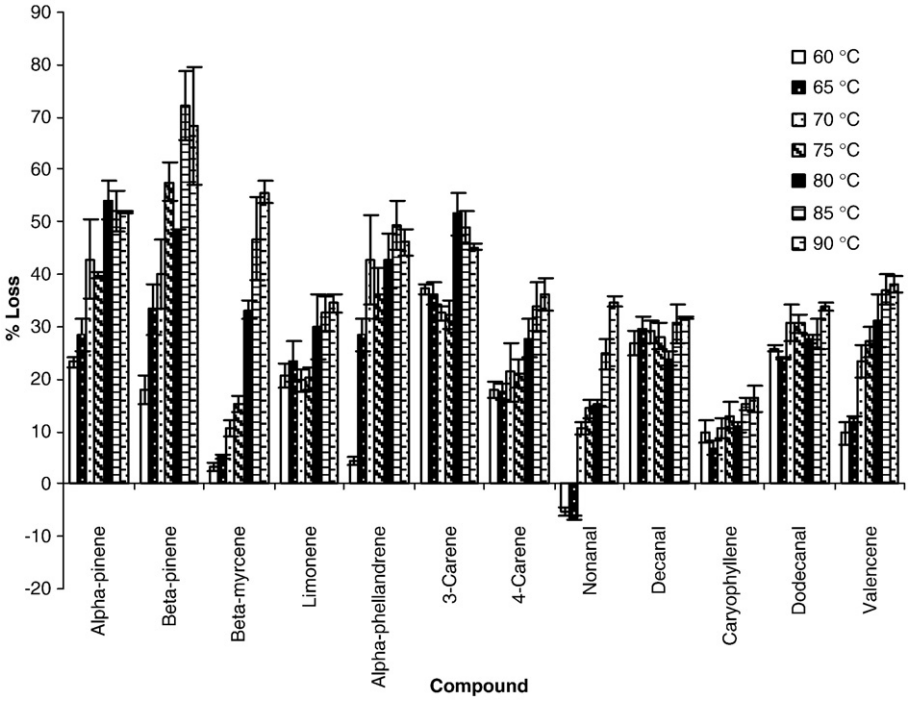

Fig. 3. Effect of thermal treatment for 1 min on the volatile compounds content in orange juice–milk based beverage.

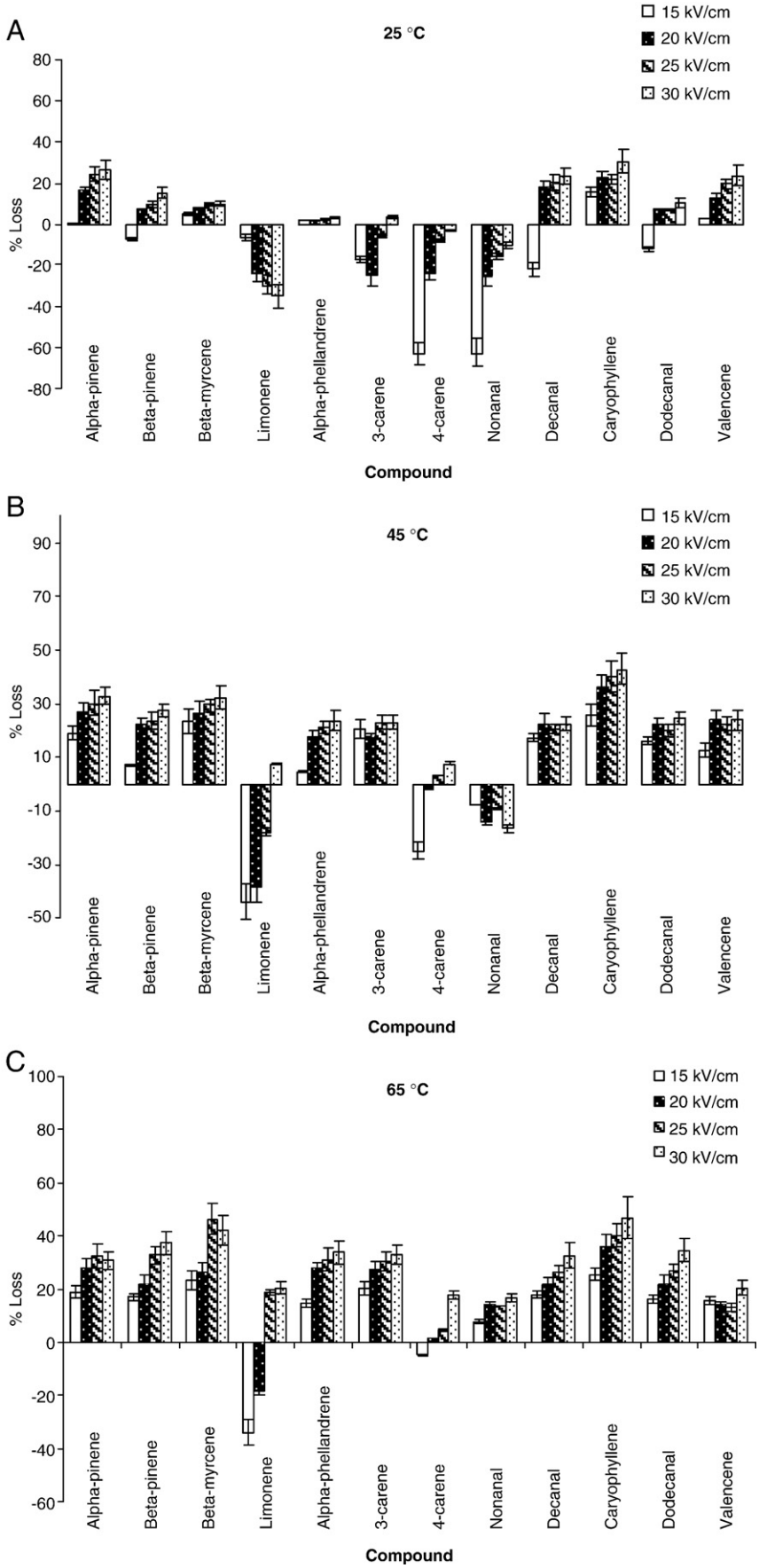

Fig. 4. Effect of combined thermal and PEF treatment (A—25, B—45 and C—65 °C) for 50 μs on % loss of volatile compounds content in orange juice–milk based beverage.

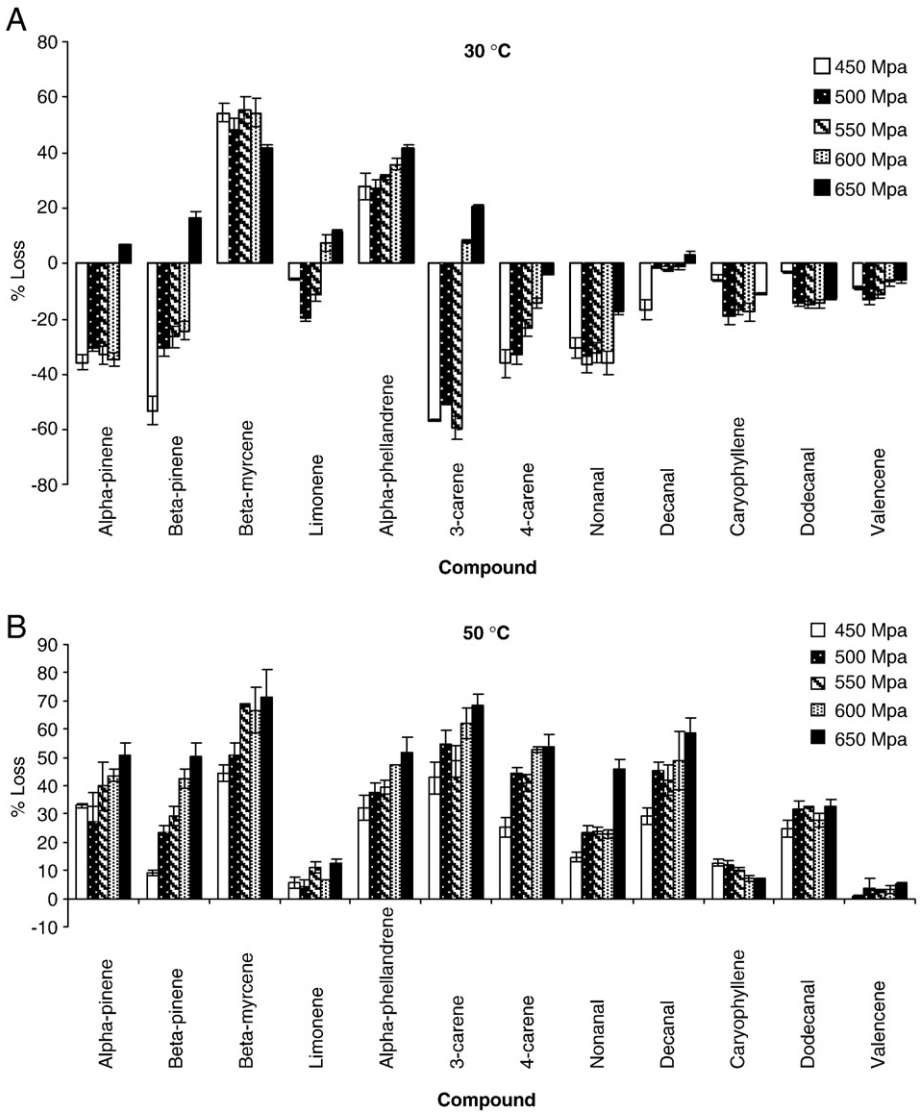

Fig. 5. Effect of combined thermal and HHP treatment (A—30 and B—50 °C) on volatile compounds content in orange juice–milk based beverage.

(~50%) and limonene, α-pynene, β-pynene, 3-carene and α-phellandrene were not affected at 650 MPa. On the other hand, the rest of components in the OJMB increased in their contents after treatment with lower pressures. Similar to the PEF treatment, it seems that the HHP treatment released several compounds that are found in the solid phase of the orange juice. At 50 °C (final temperature 65 °C) the average loss was increased in all compounds (32.80%). At 650 MPa and 50 °C the loss of volatile compounds was the same as the maximum which was reached after the thermal treatment (85–90 °C). Valencene, limonene and caryophyllene were the compounds less sensitive to the HHP treatment whereas β-myrcene, α-phellandrene and 3-carene at 50 °C were more sensitive to the HHP treatment.

The combination of different nonthermal technologies and thermal treatment (PEF at 65 °C initial temperature and HHP at 50 °C initial temperature) inactivated 90% of PME whereas high temperature treatments (85–90 °C) were needed for thermal treatment. At this temperature the loss of different volatile compounds were significantly higher than PEF and similar to HHP at 50 °C. Concentrations of several compounds were enhanced after PEF and HHP treatment at low temperatures. The sensitivity of volatile compounds differed with the different treatments applied. The high-molecular-weight compounds were more resistant to the thermal treatment and the pulp-related compounds were more resistant to the PEF and HHP treatments. Based on these results it is possible that PEF treatment can achieve a similar PME inactivation than thermal processing with a better orange juice–milk beverage fresh aroma.

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